

EXHIBIT 4

V. 2009.7.0 Revision for Sensory Evaluation of Foods, Lawless, H. T. and Heymann, H.

Chapter 6

Measurement of Sensory Thresholds

Contents:

- Introduction: The threshold concept
- Type of thresholds
- Practical methods: Ascending forced choice
- Suggested method: Ascending Forced-choice method of limits
 - Case study/Worked example
- Other Forced-choice procedures
- Probit analysis
- Sequential effects, sensory adaptation and variability
- Alternative approaches: Rated difference; Adaptive procedures; Scaling
- Dilution to threshold:
 - Odor units and Gas-Chromatography Olfactometry (GCO); Scoville units
- Conclusions

Chapter 6, Thresholds

stimulus at high levels. Such an experience, if unpleasant (such as a bitter taste), might even cause them to quit the test. On the downside, the introduction of a stopping rule can raise the false positive rate. We can think of a false positive as finding a threshold value for an individual that is due to guessing only. In the most extreme case, it would be a person who is completely insensitive (e.g. anosmic to that compound if it is an odor threshold) finding a threshold somewhere in the series. With an 8-step series, for the ASTM standard rule (everyone completes the series), the probability of finding a threshold somewhere in steps one through eight, for a completely anosmic person who is always guessing is 33.3%. For the three-in-a-row stopping rule, the chances of the anosmic person making three lucky guesses in a row somewhere rises above 50%. The sensory professional must weigh the possible negatives from exposing the participant to strong stimuli against the increased possibility of false positives creating a low threshold estimate when using a stopping rule.

Case Study/Worked example.

For the ascending forced choice method of limits (ASTM E-679), we can use a published data set for odor thresholds. The actual data set is reproduced in Appendix 6.1 at the end of this chapter. The data are from a study conducted to find the odor detection threshold for methyl tertiary butyl ether (MTBE), a gasoline additive that can contaminate ground water, rendering some well waters unpotable (Stocking et al., 2001). The ASTM procedure was followed closely, including the triangle test instructions (choose the sample different from the other two), using the 3-AFC in eight concentration steps differing by a factor of about 1.8. Individual best estimates were taken as the geometric mean of the last step missed and the first step answered correctly, with all higher steps also correct. Individuals who got the first and all subsequent steps correct (there were 10/57 or 17.5% of the group) had their estimated threshold assigned as the geometric mean of the first concentration, and the hypothetical concentration one step below that which would have been used had the series been extended down. A similar extrapolation/estimation was performed at the high end for persons that missed the target on the eighth (highest) level.

The geometric mean of the individual threshold estimates across a panel of 57 individuals, balanced for gender and representing a range of ages, was 14 ug/l (14 ppb). Figure 6.5 shows the graphical solution, which gives a threshold of about 14 ppb, in good agreement with the geometric mean calculation. This is the interpolated value for 66.7% correct, the chance-adjusted level for 50% probability of detection in the group. Confidence intervals (C.I.) for this level can be found by constructing upper and lower 95% confidence interval curves, based on the standard error of the proportion. These two curves form an envelope of uncertainty around the fitted curve. The standard error is given by the square root of $(p(1-p)/N)$ or in this case 0.062 for $p = 1/3$ and $N = 57$. The 95% C.I. is found by multiplying the z-score for .95 (= 1.96) times the standard error, in this case equal to $\pm 0.062(1.96)$ or ± 0.122 . Constructing curves higher and lower than the observed proportions by this amount will then permit interpolation at the 66.7% level to find concentrations for the upper and lower C.I. bounds.

Note that by the graphical method, the interpolated value for 10% detection (= 40% correct by Abbott's formula) will be at about 1 - 2 ppb. Similarly the interpolated value for 25% detection (50% correct by Abbott's formula), will be about 3 ppb. These values are potentially useful to a water company who wanted to set lower limits on the amount of MTBE that could be detected by proportions of the population below the arbitrary threshold value of 50%.

Other Forced Choice Methods

Ascending forced-choice procedures are widely used techniques for threshold measurement in the experimental literature on taste and smell. One early example of this approach is in the method for determining sensitivity to the bitter compound phenylthiourea, formerly called phenylthiocarbamide or PTC and the related compounds 6-n-propylthiouracil or PROP. Approximately one-third of Caucasian peoples are insensitive to the bitterness of these compound, as a function of several mutations in a bitter receptor that usually manifests as a simple homozygous recessive status for this trait (Blakeslee, 1932; Bufe et al. 2005). Early researchers felt the need to have a very